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Viruses in Groundwater

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16. ABSTRACT

The microbial contamination of ground water is a serious problem that can result in large outbreaks of waterborne disease. The purpose of this article is to review the literature available on viruses in ground water in order to evaluate the present state-of-knowledge, assess the accuracy of current standards and monitoring practices, and make recommendations for further areas of research. Reports on virus isolation from various ground-water sources are discussed with an emphasis being placed on land treatment sites. When effectively operated, land treatment can reduce viruses to a level that is achieved only with costly advanced wastewater treatment and disinfection.

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Viruses in groundwater

Bruce H. Keswick
Charles P. Gerba

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Viruses in groundwater

Their entry into aquifers, survival, migration, new methods of detection, concrete instances of contamination, and future research needs are discussed

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Groundwater is an important resource which must be protected from contamination. Already, over 19% of the nation's water supply, upwards of 46% in some areas, is taken from groundwater sources. The use of groundwater is increasing, and by the year 2000, this proportion is expected to increase to 33% of the total water used in the U.S. (*1*).

As the demand for clean water increases and supply decreases, it is evident that artificial recharge of the present sources will become more prevalent. The recharge of groundwater sources with wastewater is an attractive alternative source of groundwater and an economically favorable method of wastewater disposal. However, care must be taken to ensure the safety of this practice by preventing contamination with disease-causing bacteria and viruses passed with human excreta.

The microbial contamination of groundwater is a serious problem that can result in large outbreaks of waterborne disease. For example, Craun (*2*) reported that from 1946 to 1977, there were 264 outbreaks and 62 273 cases of illness related to contaminated (untreated or inadequately treated) groundwater. This represents 48% and 58%, respectively. Thus, 20% of the

supply accounts for 50% of the illness.

Overflow from septic tanks and cesspools was responsible for 42% of outbreaks, and 71% of illness was caused by using untreated groundwater in nonmunicipal systems. Furthermore, 356 (65%) of 550 documented outbreaks of waterborne disease from 1946 through 1977 can be attributed to illness of probable viral etiology (hepatitis A, poliomyelitis, gastroenteritis). This number probably represents only a fraction of the actual number of virus-caused outbreaks, because of the difficulties involved in proving a viral etiology of a waterborne outbreak.

In fact, direct evidence for viral involvement in waterborne outbreaks is limited to hepatitis A, adenovirus, and, recently, to the Norwalk agent. This situation has largely been ascribable to limitations in methodology for the detection of viruses in water and sensitivity of epidemiologic methods for enterovirus diseases. However, it is important to note that viruses have been demonstrated in groundwater, drinking water, and distribution systems in only a limited number of studies. Since groundwater is often used with little or no treatment for direct human consumption, the presence of viruses should not be overlooked.

As mentioned earlier, recharge of groundwater by wastewater application, besides being a source of groundwater, can also provide an economically favorable solution to the growing problem of disposing of sewage effluents (*3*). Recharge can be accomplished by direct injection or by land application and irrigation. Several recent reviews (*4–6*) have discussed the potential health risks involved in these practices and the factors affecting survival of bacteria and viruses in soils during irrigation (*7–9*). From these reviews, it is clear that the potential exists for entry, survival, and transport of microorganisms in groundwater. However, little information is available concerning these factors, especially those that affect viruses. The purpose of this article is to review the literature available on viruses in groundwater in order to evaluate the present state of knowledge, assess the accuracy of current standards and monitoring practices, and make recommendations for further areas of research.

Virus isolation

There are only a few reports in the literature on virus isolation from groundwater because of the previous lack of sensitive detection methods. The reports can be divided into two

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groups—one which includes the isolation of viruses from groundwater sources and drinking water (Table 1) and one which includes isolations from sites where groundwater recharge, land application, or wastewater experimentation is being practiced (Table 2). The differences are ones of convenience and the prior knowledge of the source and type of pollution. It is obvious that in the first group, the presence of viruses in water is more difficult to demonstrate unless the source has been monitored closely, because the contamination is most often unexpected. Usually, samples are taken only after an outbreak of overt illness or some other event has taken place, so that the contamination is often no longer present. The difficulties in drawing etiologic conclusions from these studies have been discussed in detail by Goldfield (*10*) and Melnick (*11*).

Review of these studies provides valuable information concerning the safety of groundwater sources. Wellings et al. (*12*) reported the isolation of an echovirus 22/23 complex in 100-gal samples from a 12.2-m-deep well during an outbreak of gastrointestinal illness at a migrant labor camp in Florida. The well was located 30.5 m from a solid waste field, and was in the middle of an area bordered by septic tanks. The echovirus was isolated from sewage, from potable well water containing 0.4–0.6 mg/L residual chlorine, and from stools collected from individuals living in the camp. Furthermore, it is interesting to note the occurrence of 15 cases of hepatitis A in the camp some 6 weeks later.

Thus, the virus was probably present because of well water contamination from the septic tanks, and existed in chlorinated water in the absence of evidence of bacterial contamination. It was surmised that the chlorine was ineffective against the virus which was probably associated with solids introduced with the sewage. Even though this level of treatment reduced bacterial counts to undetectable levels, the virus survived. This raises concern about the efficacy of chlorine disinfection of contaminated groundwater, based on currently accepted standards.

Mack et al. (*13*) isolated vaccinelike poliovirus type 2 from a well responsible for a gastroenteritis outbreak at a restaurant in Michigan. Five-gallon samples were taken from the 30.5-m-deep well and were concentrated by a PE60/centrifugation technique. Coliform levels in the well ranged 0–16/100 mL, but no *Salmonella* or *Shigella* were found. The pollution

TABLE 1
Isolation of viruses from drinking water wells

Location	Virus types	Concentration method	Source	Reference
Florida	Echo 22/23	Membrane adsorption	Well	*12*
Germany	Echo 3, 6, 30; Coxsackie B1, 4, 5; U[a]	$Al(SO_4)_3$	Well	*16*
India	U	Iron oxide	Well	*18*
Michigan	Polio 2	PE60/centrifugation	Well	*13*
Israel	Echo 6, 7; Coxsackie B6; Polio 1; U	Membrane	Well	*14*
England	Polio 2	$AlCl_3$/membrane	Well	*15*
Israel	Polio 1; U	Organic flocculation	Well	*17*
Ghana	Polio 1; Coxsackie B3	Centrifugation	Well	*19*
Mexico	Rota; Coxsackie B4, 6	Membrane	Well	*50*

[a] U = unidentified.

TABLE 2
Isolation of viruses beneath land treatment sites

Site location	Type of site[a]	Virus types	Maximum distance of virus migration (m)		Reference
			Depth	Horizontal	
St. Petersburg, Fla.	S	Polio 1; coxsackie B4; echo 7	6	—	*22*
Gainesville, Fla.	S	Coxsackie B4; Polio 1, 2	3	7	*21*
East Meadow, N.Y.	R	Echo 12; U[b]	11.4	3	*23, 24*
Holbrook, N.Y.	R	Echo 6, 21, 24, 25; U	6.1	45.7	*23, 24*
Sayville, N.Y.	R	U	2.4	3	*23, 24*
12 Pines, N.Y.	R	Polio 2; U	6.4	—	*24*
North Masapequa, N.Y.	R	Echo 11, 23; Coxsackie A16	9.1	—	*23*
Babylon, N.Y.	R	Coxsackie B3; U	22.8	408	*23*
Ft. Devens, Mass.	R	U	28.9	183	*26*
Vineland, N.J.	R	Polio; coxsackie B3; echo	16.8	250	*27*
Lake George, N.Y.	R	Phage	45.7	400	*29*
Phoenix, Ariz.	R	Coxsackie B3	18.3	3	—[c]
Lubbock, Tex.	S	Coxsackie B3	30.5	—	*38*
Kerrville, Tex.	S	U	1.4	—	*40*
Dan Region, Israel	R	Polio 1, 2, 3	31–67	60–270	*37*

[a] R = rapid infiltration; S = slow rate infiltration.
[b] U = unidentified.
[c] Gerba, unpublished data.

source was a waste drain field that allowed sewage to enter the well by passing through 5.5 m of clay, 2.5 m of shale, and 22.5 m of limestone. No information on the soil structure was given.

Several points should be made here. Although poliovirus was isolated from the well, it was not suggested as the causative agent of the gastroenteritis outbreak. That outbreak was probably attributable to some other virus (such as rotavirus or the Norwalk agent) which could not be detected by the methods available at that time. Also, it is evident that soil type, structure, and proximity of a well to a source of sewage play an important role in the contamination of groundwater sources, as the virus in this case penetrated 30.5 m through several soil profiles, and traveled 91.5 m laterally.

Marzouk et al. (*14*) collected 20–440-L samples of groundwater from 3-m-deep wells in Israel. Twenty of 99 samples were found to be positive for viruses. The viruses isolated included coxsackievirus B6, echoviruses 6 and 7, poliovirus 1, and several unidentified types. Total bacteria, fecal coliform, and fecal streptococcal concentrations ranged from 0–10^4, 0–200, and 0–100, respectively, per 100 mL. In at least one case, viruses were detected in samples that contained no total bacteria, while 12 of 17 samples that were negative for fecal coliforms and fecal streptococcus were positive for viruses. No statistical correlation could be demonstrated between indicator bacteria and the presence of viruses, which suggests the failure of bacterial indicators to represent viral contamination adequately.

Morris (*15*) isolated poliovirus type 2 in two samples of groundwater in England. Concentrations of 1 plaque-forming unit (PFU)/40 L and 4 PFU/10 L were detected in these 5–100-L samples. This well was to be used untreated for direct human consumption, but, subsequent to these findings, was shut down.

Walter and Rudiger (*16*) found that eight of 62 10-L samples from a groundwater source for a large water supply system contained viruses. Echoviruses 3, 6, and 30; coxsackieviruses B1, B4, and B5; and several unidentified viruses were recovered. No coliform data were given in the report.

Shuval (*17*) reported the isolation of poliovirus from a well under suspicion during an outbreak of hepatitis A in Israel. Poliovirus type 1 (4.0–7.2 PFU/L) was recovered from a single well, as were coliform bacteria.

Rao et al. (*18*) detected viruses in six different wells in Nagpur, India. Concentrations of 2–8 PFU were recovered from 40-L samples collected at depths of 12–24 m. No attempt was made to identify the isolates.

Addy and Otatume (*19*) isolated poliovirus 1 and coxsackievirus B3 from three of eight wells in the greater Accra region in Ghana. Those wells were dug specifically to provide water for human consumption. Also, a report from China (*20*) indicates that waterborne hepatitis traceable to well water contamination has caused several large epidemics.

The studies above illustrate the need for monitoring and maintaining the integrity of groundwater sources. It is obvious that, in many cases, groundwater assumed to be safe is not. This and other implications of these studies will be discussed later.

Land application sites

Even though there have been no reports of disease outbreaks associated with land treatment of sewage wastes, there are a growing number of studies concerning the detection of viruses in groundwater after wastewater application to land or direct groundwater recharge. These studies point out questions, discussed later in this article, which must be answered in order to ensure the safety of these practices.

Wellings et al. (*21*) demonstrated vertical and lateral movement of virus in secondary effluent discharged into a cyprus dome (a wetland ecosystem). Poliovirus 1, coxsackievirus B4, and echoviruses 7, 11, and 14 were recovered from 3-m-deep wells in three of 71 samples, at concentrations ranging 4–353 PFU. Viruses migrated 7–38 m laterally from the application point and survived at least 28 days.

The soil at this site ranged from a top 0.6-m layer of black organic soil (4–12% clay) to a sandy clay and a solid blue clay with a permeability of 3×10^{-2} cm/min to 3×10^{-6} cm/min. Thus, the viruses moved horizontally as well as vertically and survived many days under ambient conditions, indicating a necessity to evaluate such sites for their aquifer movement and transmission of viruses to drinking water sources.

In an earlier study, Wellings et al. (*22*) recovered viruses from groundwater after spray irrigation of secondary sewage effluent onto an Imlokee sand (little or no silt or clay). Of particular interest in this study was that viruses survived chlorination, sunlight, spraying, and percolation through 3–6 m of sandy soil; furthermore, after a period of heavy rains, a burst of viruses was detected in samples that had previously been negative. These studies demonstrate that soil type, rainfall, and other factors can affect viral movement into groundwater, and that viruses are capable of surviving long periods which, when combined with the ability to move long distances laterally, could lead to wide dispersal through an aquifer.

Vaughn and Landry (*23, 24*) and Vaughn et al. (*25*) reported isolations of viruses from four groundwater recharge sites, from a stormwater recharge basin, and from groundwater under a sanitary landfill in New York. These sites have soils of coarse sand, fine gravel, and 1–2% silt. At the groundwater recharge sites, viruses were recovered at depths up to 11.4 m and at distances up to 45.7 m from the injection point of secondary or tertiary chlorinated effluent. As much as 22–33% of the 100-gal samples at the four sites were positive for viruses, with concentrations of 1.3–10.6 PFU/gal. In addition, total coliforms and fecal coliforms were found in these samples. In order to reach the groundwater, viruses moved through 5.5–9 m of the overlying soil.

Moreover, at the 12 Pines site, viruses were discovered in groundwater under basins where effluent seeded with viruses was applied at rates of 6–100 cm/h. The slower infiltration rates were more effective in removing the viruses, suggesting that site management is important.

Both the landfill and stormwater recharge basin also yielded viruses. At the landfill site, viruses were detected at depths of 22.8 m and up to 408 m downstream. Coxsackievirus B3 and other unidentified viruses were detected. At the stormwater recharge site, samples taken at 9-m depths directly in the basin were positive for echoviruses 11 and 23 and for coxsackievirus A6. This contamination may have originated from runoff from cesspools in the area.

Schaub and Sorber (*26*) reported on a study of viruses in groundwater under rapid infiltration cells at Ft. Devens, Mass. The soil consisted of silty sand and gravel underlain by bedrock. The groundwater contained viruses at depths of 29 m and lateral distances of 183 m, with concentrations of about 8.3% of the applied effluent. Fecal streptococcal bacteria were also found in the 28.9-m-deep well. Marker F2 virus was applied at this same site; only about 50% of the virus was removed, and it was detectable for at least 11 days. This site was deemed to have poor filtration prop-

erties, and points out the need for site-specific evaluation.

At the Vineland, N.J., rapid infiltration site (*27*) primary effluent was applied to Cohansey sand and coarse gravel. Viruses were found at 16.8-m depths and up to 250-m lateral distances in 19 of 40 samples. Polio-, echo-, and coxsackieviruses were identified. Total coliforms and fecal coliforms were found consistently at depths up to 6 m beneath the recharge basins. Total coliforms also occasionally occurred at greater depths and downstream. In contrast, no fecal coliforms were found in samples taken below 9.1 m and coliforms occurred only once in a shallow well 50 m down-gradient. Thus, viruses penetrated deeper into the ground and moved longer distances than did the coliforms.

The potential for viruses to migrate great distances, as in the previous study, was further demonstrated by Noonan and McNabb (*28*), who used the phages ϕX174 and T4 to demonstrate lateral movements of 140 m and 911 m, respectively, in New Zealand groundwater in just 96 h. The viruses moved at greater than 300 m/d, and survived for at least seven days. In laboratory studies, 6.2 days were necessary for a 90% reduction in titer; so in this case, the viruses could theoretically travel at least 2.5 km in groundwater before a 90% reduction could be effected under these conditions.

Aulenbach (*29*) reported isolating coliphage from groundwater under the Lake George, N.Y., recharge basin in a very limited study. No conclusive information on viruses can be determined from this study because control samples also yielded phage. Apparently, coliforms were detected in only some of the wells, whereas coliphage was detected in all of the wells. Samples collected after a period of heavy rainfall had higher counts. The effluent at this site was applied to sand beds and monitoring wells were located at various distances (up to 400 m) and depths (up to 45.7 m) in and around the recharge basins.

Septic tank effluent seeded with coxsackieviruses A5 and A14 was found to pass through sand beds and into groundwater in a study in the U.S.S.R. Grigoryeva and Goncharuk (*30*) reported that the viruses survived for 20 days under these conditions. Others (*31, 32*) have also reported on the movement of bacteria and viruses from septic tanks to groundwater, which may be a significant problem in rural areas, as indicated by a recent groundwater quality survey (*33*).

Viruses in groundwater at other recharge sites have been studied with varying success. At the Flushing Meadows site near Phoenix, Ariz. (*34*), it was found that a fine loamy sand over coarse sand and gravel effectively removed viruses. Laboratory studies confirmed that this soil was an excellent adsorber. No viruses were detected in any of the samples of renovated water, even though levels of 158–475 PFU/mL were detected in the effluent applied. However, coliform organisms were detected in the renovated water, suggesting that the removal mechanisms must have been different for viruses and bacteria, and that viruses may have been present. Since this site is no longer in existence, these findings cannot be confirmed. However, since then, virus has been detected in a sample from an 18.3-m-deep well at a nearby land application site. The isolate was identified as coxsackievirus B3.

Initial findings at the Dan Region (*35, 36*) reclamation project in Israel yielded negative results when 10-L volumes were sampled. However, when the sample size was increased to 400 L, viruses were detected (*37*), which emphasizes the need for large sample volumes.

At two land treatment sites where sewage is used to irrigate cropland, both positive and negative virus isolations have been made (*38, 39*). At the Lubbock, Tex., site, coxsackievirus B3 was isolated from a depth of 30.5 m; at Roswell, N.M., no virus isolates were detected in samples taken from 3–30-m depths. In the latter case, irrigation is seasonal and intermittent, whereas application at the Lubbock site is continuous.

At an operational land application site in Kerrville, Tex. (*40*), no viruses were detected in the monitoring wells at depths of 10.7–19.8 m even though viruses could be detected in 13.7-m-deep lysimeters. Depth to groundwater was an important factor in this study.

In one often-cited report (*41*) on the Santee project, no viruses were detected in renovated water. This is not surprising, since the detection methods available at that time were not quantitative. These negative results must therefore be considered highly questionable, as should the results obtained at the Whittier Narrows, Calif. (*42*), and the San Angelo, Tex. (*43*), projects, which did not employ techniques sensitive enough to detect low levels of virus. This reiterates the need for careful evaluation of methods used in any report before negative conclusions are accepted.

Survival factors

It is apparent that the factors affecting virus survival in groundwater are complex and poorly understood (Table 3, Figure 1). To control the many variables present in the field, the movement of viruses through soils to groundwater has been modeled in the laboratory by the use of soil columns. These studies have recently been reviewed (*7–9*), and the details will not be repeated here except to support field data.

Virus type. Laboratory studies have shown that viruses have widely differing adsorption properties. This is therefore an important factor in the movement of viruses from sources of contamination, such as septic fields, into otherwise clean groundwater. Thus, for evaluation of a site, several types of viruses should be tested in order to ensure a representative picture. Furthermore, even with the development of new concentration methods, it is still difficult or impossible at this time to detect all of the more than 100 types of human enteric viruses. For example, there is presently no readily available system for cultivating hepatitis A virus; until recently, this was also true of the rotaviruses. Thus, some viruses may be present but remain undetected or unidentified.

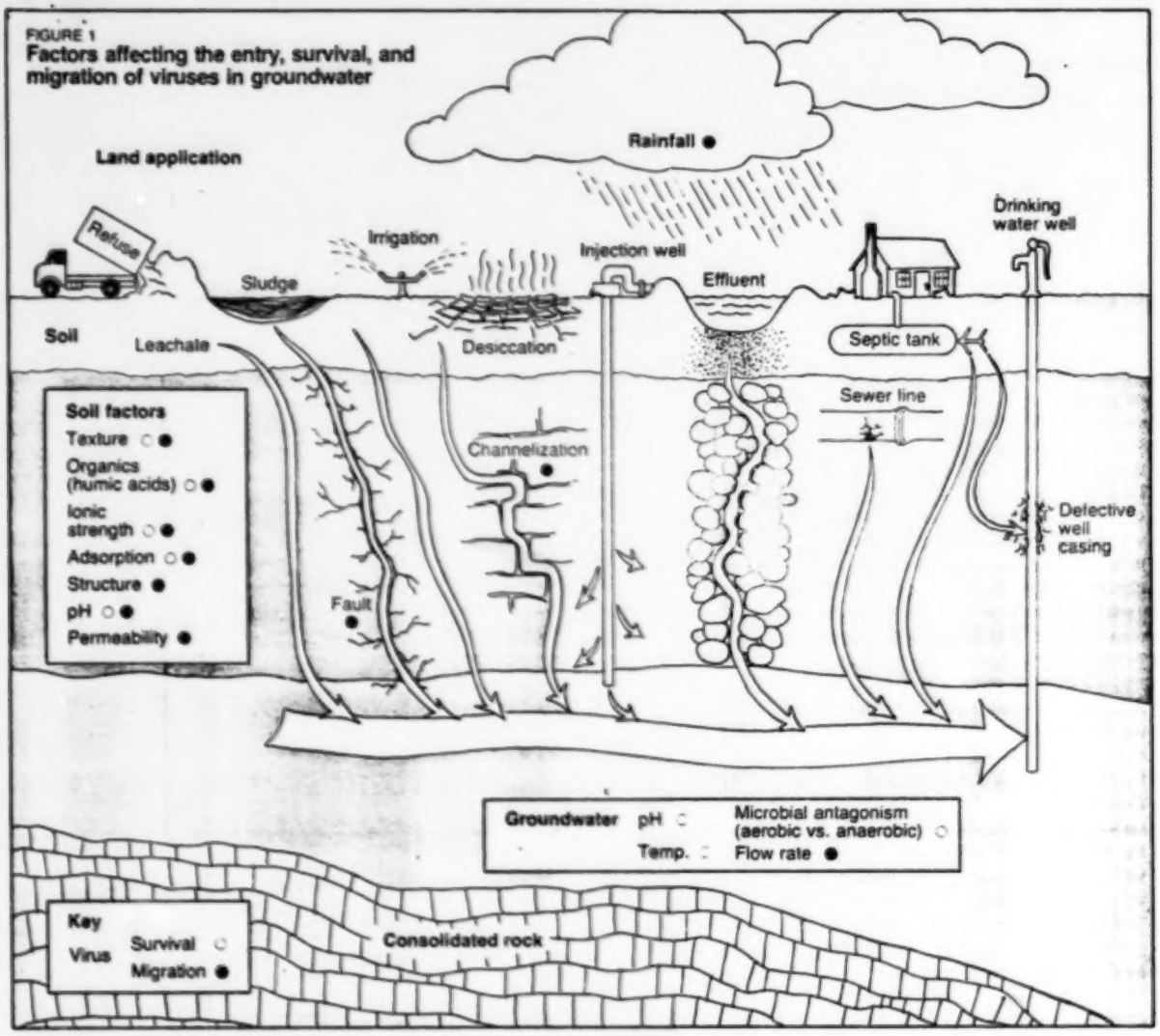
Soils. Obviously, soils vary considerably both in their composition and in

TABLE 3
Factors affecting survival and migration of viruses in groundwater

Factor	Survival	Migration
Hydrogeological		
Soil		
Texture (sand, silt, clay)	+[a]	+
Organics	+	+
Humic acids	?	+
Cations	+	+
Adsorption	+	+
pH	+	+
Ionic strength	0	+
Permeability	0	+
Biological		
Virus type	+	+
Microbial antagonism	+	0
Meteorological		
Rainfall	+	+
Temperature	+	0
Desiccation	+	+
Sunlight	+	0

[a] + = affects survival or migration; 0 = no effect; ? = effect unknown.

FIGURE 1
Factors affecting the entry, survival, and migration of viruses in groundwater



their overall structure. Laboratory studies have shown the importance of the composition of soils, including the organic content; percent sand, silt, and clay; pH; cation-exchange capacity; permeability; texture; moisture; and iron oxide levels.

As pointed out in earlier reviews, each of these features can effect virus removal by soils, and so the study of this problem becomes quite complex. For example, virus removal by the soil types in the studies reviewed here ranged from very poor to apparently total removal, which indicates the need for site-specific evaluations of soil properties. The variability of adsorption by different soils has been repeatedly demonstrated in the laboratory (*7*, *8*, *44*, *45*). In general, sandy and organic soils are poor adsorbers and clay soils are good adsorbers.

Subsurface structure has also been demonstrated to be an important promoter of groundwater contamination. For example, movement of bacteria through fracture zones was shown by Allen and Morrison (*46*) to be rapid and to follow the fracture lines, leading to rapid and wide dispersal. Thus, under natural conditions, soil columns are not always indicative of the ability of pollutants to enter groundwater. Furthermore, the columns used in many studies were too short, and may have overestimated virus removal rates. There is now evidence (*47*) that removal rates in subsurface layers are lower than at the surface. So estimates based on the faster rates observed in short columns are higher than those found in columns longer than 50 cm.

Virus survival in groundwater. It is difficult to make firm assessments on virus survival in natural waters because viral resistance to factors that influence their survival varies. In addition, these factors have shown great variation temporally, which makes predictions even more difficult. For instance, little is known concerning survival rates of human enteric viruses in soil and groundwater. Field studies by Wellings et al. (*21*) suggested that viruses could survive for at least 28 days in groundwater. Survival of viruses in surface waters for as long as 188 days has been reported (*48*), while in other laboratory experiments viruses survived more than 200 days in drinking water. Since the effects of sunlight are eliminated and the temperature is lower, even longer survival times would be probable in groundwater.

Recent studies in our laboratory indicate that virus survival in soil is probably controlled by the principal factors shown in Figure 1. An evaluation of these factors indicates that because of the detrimental effect of aerobic soil microorganisms, evaporation, and higher temperatures, inactivation of viruses will be much more rapid near the surface than for those viruses

that penetrate the soil more deeply. Thus, viruses which penetrate the soil surface are expected to survive for prolonged periods of time, as compared to those retained near the soil surface. The factors which affect virus survival in soils are complex and should be described kinetically in terms of initial populations and survival rates, as well as duration. These factors have been reviewed in detail (*7–9*).

Migration of viruses in soils and groundwater. The studies by Wellings et al. (*12, 21, 22*) and Mack et al. (*13*) were the first to demonstrate transport of viruses for long distances through soils into groundwater. Prior to their studies, it could be concluded only on epidemiologic evidence that viruses (mainly hepatitis A) move from sources of contamination into well water some distance away (*49*). Now, it is generally accepted that once in groundwater, viruses can migrate great distances under the right condition such as those found in the study by Noonan and McNabb (*28*).

However, the specific factors affecting virus movement in groundwater have not been studied directly. As mentioned above, there are many factors, such as the degree of saturation, subsurface flow, hydrogeologic structure, and the like, involved in this movement which are site-specific and which need to be evaluated for any site where wastes are to be applied.

Groundwater also can be contaminated by both purposeful (recharge) and accidental (septic or sewage leakage) events. The extent of influence of this man-made factor is evidenced by a recent survey (*33*) of groundwater quality, which indicated that 92.5% of the rural samples taken were positive for total coliforms. In many of the 460 samples, contamination was related to depth and distance from septic tanks. In view of the high bacterial counts in the wells sampled, it is very likely that viruses were also present. Well contamination should be preventable to a large degree by proper location, construction, and maintenance, with an additional measure of safety provided by disinfection. However, as was seen, chlorination of viruses, especially when they are associated with solids, can be unreliable. Hence, extra care should be taken in the location of wells and septic tanks.

Methodology. The last 10 years have seen great progress in the development of virus detection methods. Their absence from earlier studies renders any negative findings unreliable, as the levels of virus detectable by older methods were not as sensitive as today's methods. Furthermore, it is still not possible to detect hepatitis A and many agents of viral gastroenteritis routinely, although work in this area is progressing; and only recently has it become possible to detect rotaviruses in water samples (*50*). Moreover, the methods for virus isolation fail to detect the presence of coxsackievirus group A, adenoviruses, and reoviruses unless additional efforts are undertaken. Methods are available for less than half of all the viruses known to be present in human wastes. Given these limitations, it is very doubtful that more than 1% of all the viruses present in domestic wastes are detected by the routine methodology currently in use.

Operation of land application sites. Using soil column models, Gerba and Lance (*51*) found that the degree of viral movement and removal by soils from a land application site could be controlled to some degree by the way in which the basins were flooded with sewage in relation to the rainfall patterns of the site. Others (Table 4) have mentioned operational features that may also have an effect, and it is apparent that these factors need to be taken into account in the management of a refuse site.

TABLE 4
Land treatment practices that may limit groundwater contamination by viruses

Practice	Comment	Reference
Drying	Reduces virus movement by enhancing virus inactivation	*55*
Flooding with wastewater after rainfall	Reduces virus movement	*49*
Infiltration pattern	Shorter flooding/drying cycles limit virus penetration	*56*
Infiltration rate	Slower rate promotes virus removal	*25*
Site evaluation	Nature of soil, depth to groundwater, and substructure assures adequate virus removal	*9, 26, 40*

TABLE 5
Research needs

Basic research
- Virus interaction with surfaces
- Virus inactivation and degradation in groundwater
- Virus interaction with organics in natural waters

Methods development
- Tracers
- Laboratory methods for determining virus retention by soils
- Groundwater and soil sampling
- Predictive models

Criteria
- Adequacy of bacterial standards for groundwater quality
- Acceptable removal of pathogens by land application of wastewater
- Zoning of aquifers for land treatment of wastewater

Renovation and protection of aquifers
- Soil modification for enhanced removal of viruses
- Enhancement of aerobic microflora
- Management practices for groundwater recharge operations, i.e., duration of flooding and drying cycles, etc.

Field studies
- Land treatment sites
- Septic tanks
- Disease outbreaks by groundwater contamination
- Survival time of viruses in groundwater

Research recommendations

As is apparent from this review, current information on viruses in

groundwater is limited. Our knowledge in certain areas is especially deficient. Filling these gaps will require the utilization of current methodology and the development of new methods for virus surveys of groundwater (Table 5). Such surveys should focus on rural private sources and small communities where water is used with minimal or no treatment.

Results of previous surveys on the bacteriological quality of groundwater indicate that viral pollution is more widespread than might be believed. Furthermore, many of the previous systems used for virus concentration had efficiencies of 2-3%, and were limited to the detection of enteroviruses (*52*). Now, however, systems with efficiencies of 30% and greater are available (*50*).

Viruses are known to enter groundwater under certain conditions, but these conditions have not been adequately defined. It is not yet possible to determine exactly what factors affect the retention and mobility of viruses in soils. Such an understanding is essential so that predictions may be made and methods may be developed for estimating virus migration.

The abilities of viruses to survive in groundwater and to migrate long distances have been described in this review. However, studies are needed on the survival of additional types of viruses actually suspended in groundwater under field conditions. One approach would be to develop survival chambers similar to those used by McFeters to study bacterial survival in surface and well waters (*53*). This is especially important where direct recharge is to be practiced, because the length of holding or turnover time could affect virus survival.

The factors which promote or are detrimental to virus survival should also be studied in the laboratory. For example, it has been found that association with solids, organic material, pH, and temperature may influence virus survival in groundwater. Microorganisms are known to travel in groundwater at different rates from those of commonly used dyes (*54*). Studying potential migration in groundwater thus requires the development of tracers that will reflect virus behavior in the subsurface.

Also, human viruses can easily be grown in the laboratory, but cannot be safely used in field studies because of possible adverse health effects. Developing bacteriophage tracers that exhibit movement and survival similar to animal viruses may be one alternative approach.

It is certain from this review that, in many instances, bacteria are not adequate indicators of viral pollution. It is questionable whether bacterial standards for surface water will be adequate for groundwater, especially since groundwater is often used without any treatment and has been responsible for a great portion of the reported waterborne disease outbreaks. New bacterial and viral standards should be developed to control groundwater pollution.

TABLE 6
Parameters for using laboratory columns to evaluate land treatment sites for virus removal

Parameter	Comment
Virus	More than one type is necessary; use both good and poor adsorbers
Soil columns	Need to be of adequate length
Application	Sewage or effluent to actually be used should be tested
	Rainfall should be simulated
	Infiltration and drying rates should be evaluated

This report is not intended to discourage groundwater recharge with sewage effluent. In fact, we hope to encourage such practices as land application, but with the caution that each site must be evaluated individually for its capacity to aid the elimination of viruses from wastewater and to limit the potential impact on groundwater quality. Moreover, when effectively operated, land treatment can reduce viruses to a level that is achieved only with costly advanced wastewater treatment and disinfection. In addition, great energy savings as well as elimination of the production of potentially carcinogenic substances can be achieved.

It is recommended, therefore, that laboratory studies be conducted to determine the virus-soil interactions of a particular site by using columns at least 50 cm in length to model the site (Table 6). Varied patterns of wastewater application and climatological factors such as rainfall should be simulated. In addition, viruses which have been shown to be both good and poor adsorbers should be tested.

Also, studies should be conducted on soil modification (addition of small amounts of substances such as iron oxides, clays, and so on) designed to enhance virus removal. Hopefully, from this information, it may be determined in advance whether or not the soil is suited to the type of application desired, and how management practices or soil treatment could make it more suitable. Of course, these studies cannot perfectly simulate the actual field site; thus, it is further recommended that any groundwater subject to recharge (as under a land application site) be monitored on a regular basis for the presence of virus. Several times a year should be adequate, unless virus levels are likely to be high, and the intended purpose of the water, such as direct reuse, warrants more careful monitoring.

The influence of physical and environmental factors (fractures, rainfall, etc.) should also be studied. For example, groundwater should be examined for virus after periods of heavy rainfall, to see if previously adsorbed virus is being eluted and progressing into the aquifer. Differences in the type or pattern of application should also be followed closely. In short, it is deemed important to evaluate each site thoroughly and individually, with regard to viruses before one of our valuable groundwater sources is damaged by contamination.

Both field and laboratory studies are also greatly needed to aid in the design and management of septic tanks. For instance, to our knowledge, no field studies using modern virus concentration technology have been conducted on virus occurrence in the vicinity of septic tank drainfields.

Recent advances in the methods of concentrating and detecting viruses in water have continued to reduce the cost of collection and analysis for virus monitoring. Continued efforts in these areas should be made so that the monitoring can be more generally feasible for all concerned with viral contamination of the environment.

The presence of viruses in groundwater obviously requires further study. The results obtained from these studies will be helpful in planning and operating land application sites and septic tank drainage fields. This will in turn, directly benefit the public by providing the information necessary to protect valuable groundwater resources from microbial contamination, and, hopefully, will help reduce the waterborne transmission of infectious diseases.

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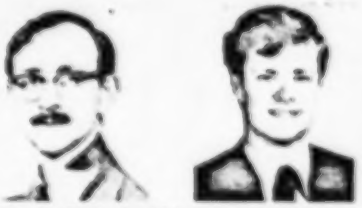

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